Brief Definitive Report

Human Histocompatibility Leukocyte Antigen (HLA)-DM Edits Peptides Presented by HLA-DR According to Their Ligand Binding Motifs

By S. Marieke van Ham,* Ulrike Grüneberg,* Georg Malcherek,‡ Iris Bröker,* Arthur Melms,‡ and John Trowsdale*

*From *Human Immunogenetics Laboratory, Imperial Cancer Research Fund, Holborn, London WC2A 3PX, United Kingdom; and ‡Neurologische Klinik, Universitat Tubingen, 72076 Tubingen, Germany*

Summary

Human histocompatibility leukocyte antigen (HLA)-DM is a facilitator of antigen presentation via major histocompatibility complex (MHC) class II molecules. In the absence of HLA-DM, MHC class II molecules do not present natural peptides, but tend to remain associated with class II–associated invariant chain peptides (CLIP). Recently, DM was shown to catalyze the release of CLIP from HLA-DR. We have investigated which peptides bound to HLA-DR are vulnerable to release upon encountering DM. By directed substitution of allele-specific anchor residues between CLIP and DR3–cognate peptides and the application of recombinant DM we show that DM catalyzes the release of those peptides bound to HLA-DR3 that do not have appropriate anchor residues and, hence, no optimal ligand binding motif. Thus, HLA-DM acts as a peptide editor, facilitating selection of peptides that stably bind to class II molecules for eventual presentation to the immune system from the pool of available peptides.

MHC class II molecules are heterodimeric transmembrane glycoproteins that present peptides to CD4 Th cells (1, 2). HLA-DR, a prototype class II molecule, usually presents peptides derived from exogenous sources. After assembly of the MHC class II αβ dimers in the endoplasmic reticulum (ER), the complex associates with the invariant chain (Ii) (3). Subsequently, the αβ–Ii complex is targeted to specialized antigen-processing (MIIC) vesicles that contain antigens gathered by endocytosis (4, 5). Ii is sequentially degraded, allowing class II molecules to bind exogenous peptides in the MIICs. One of the final biosynthetic intermediates in the degradation of Ii is the complex between αβDR and class II–associated invariant chain peptide (CLIP), which represents a nested set of Ii-derived peptides that bind in the groove of the class II complex. In vitro experiments show that release of CLIP requires an acidic pH, which is a feature of endocytic compartments in vivo (6–8).

In cell lines mutated for the MHC-encoded HLA-DM molecule, however, DR3 remains mainly complexed to CLIP, indicating failure to release this Ii peptide. Similarly, in transgenic mice lacking H2-M, the murine equivalent of HLA-DM, the majority of the class II molecules is bound to CLIP (9, 10). DM is a nonpolymorphic, αβ-heterodimeric complex located in MIIC compartments (11–16). Recently, DM was shown in vitro to catalyze the dissociation of CLIP from DR3 (17–19). Thus, DM seems to facilitate the generation of DR complexes that can accommodate antigenic peptides. Besides CLIP, DM is able to release some but not all antigenic peptides from class II molecules (17). We set out to determine which peptide–DR complexes are preferential substrates for the catalytic action of HLA-DM and which factors determine this process. We demonstrate that DM functions as a general catalyst for peptide release from DR. The composition of the bound peptide is the crucial factor determining which peptides can be released from the class II complex upon encountering DM.

Materials and Methods

Cell Lines and Culture Conditions. The B × T hybrid cell line T2.DR3, a stable derivative of the T2 cell line transfected with HLA-DR3, was kindly provided by P. Cresswell (20) and maintained in RPMI supplemented with 5% fetal calf serum and 500 μg/ml G418 (Sigma, Poole, UK). Sf9 cells (PharMingen, San Diego, CA) were grown in suspension in Grace's medium (Imperial Laboratories, London, UK) supplemented with 10% fetal calf serum, 1% Amphotericin B (Sigma), and 50 μg/ml Gentamycin (Sigma) at 37°C.

mAbs and Peptides. mAb L243 (American Type Culture Collection, Rockville, MD) binds to a nonpolymorphic determinant present on HLA-DR molecules. mAb 5C1 was raised against the α1 and α2 domains of DMα as described before (21). The DMβ-specific antiserum FS4 was raised by F. Sanderson (Imperial Cancer Research Fund, London, UK) by immunizing rabbits with recombinant DMβ protein expressed in bacteria. His-tag–specific mAb was obtained from Dianova.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/96/11/2019/06 $2.00
Volume 184 November 1996 2019–2024

Table 1. *Alignment of Peptides According to the HLA-DR3 Binding Motif*

Antigen	Sequence	Relative position 1	4	6	9
CLIP (81–104)	LPKPPKPVSKMRMATPLLMQALPM				
CLIP (81–104) A→D	LPKPPKPVSKMRMDTPLLMQALPM				
CLIP (89–101)	SKMRMATPLLMQALPM				
CLIP (89–101) P→K	SKMRMATKLLMQALPM				
CLIP (89–101) A→D, P→K	SKMRMDTKLLMQALPM				
CLIP (81–92)	LPKPPKPVSKMR				
ApoB (2877–2894)	ISNQLTLDSNIKYFHKLN				
ApoB (2877–2894) D→A	ISNQLTLASNIKYFHKLN				
HAChRα (312–325)	VRKVFIDTIPNIME				
MOMP (251–265)	QASLALSYRLNMFTP				

Amino acids forming optimal specific contact sites for the HLA-DR3-binding groove are indicated in bold and nonoptimal specific contact sites are underlined (23, 29). Peptides are referenced in 19, 23, 25, 29.

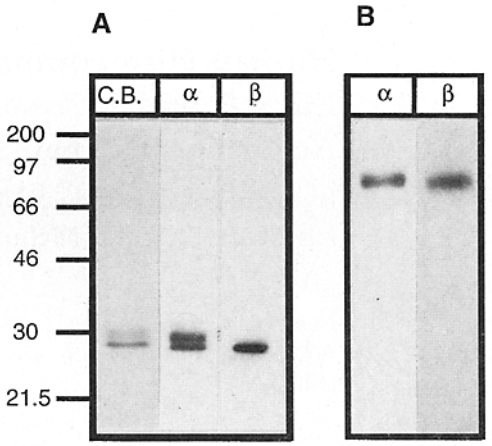

Figure 1. Recombinant soluble DM. Affinity purified sDM molecules were subjected to PAGE on SDS gels (*A*), followed by Coomassie blue staining (lane *C.B.*) or Western blotting (lanes α and β). Two differently glycosylated DMα chains were detected by the DMα-specific mAb 5C1 (21) (lane α) and the DMβ chain was detected by a mAb specific for the His-tag on the DMβ chain (lane β). Molecular sizes are indicated on the left (in kD). Analysis of purified sDM on a native gel (*B*) was followed by Western blotting using the mAb 5C1 for the DMα chain (lane α) or the DMβ-specific antiserum FS4 (lane β).

Peptides (Table 1) were synthesized on a multiple peptide synthesiser (SMPS 350, Zinsser Analytic, Frankfurt/M, Germany) using Fmoc/tBu strategy and Wang resin (*p*-benzyloxybenzylalcohol-polystyrene). NH_2-terminal biotinylation was conducted during synthesis and peptides were checked for purity and structure as described (25).

Production and Purification of HLA-DM and HLA-DR. Truncated genes coding for soluble DMα and DMβ chains lacking the transmembrane and the cytosolic regions were generated by polymerase chain reaction. The genes were truncated after codons 244 (DMA) and 274 (DMB) of the original cDNA sequences (13) and a hexa-histidine tag (His-tag) was genetically attached to the novel COOH terminus of the DMβ chain. The constructs were cloned into the dual promoter transfer vector pBacp10pol(22) and recombinant baculoviruses were produced using BaculoGold DNA (PharMingen). sDM was produced using Sf9 cells in protein-free medium (PharMingen) to prevent aggregation of the recombinant protein. sDM was purified by affinity chromatography via the His-tag using TALON metal affinity resin (Clontech, Palo Alto, CA) in the presence of 1% detergent ($C_{12}E_9$ or NP-40). The complex was eluted using a gradient of 0–100 mM imidazole in PBS, pH 8.0.

HLA-DR3 molecules were purified from T2.DR3 cells by affinity purification using L243-coupled CNBr-activated Sepharose (Pharmacia, Milton Keynes, UK), essentially as described before (23). The complexes were eluted with 25 mM Na_2CO_3, 0.15 M NaCL, 0.1% $C_{12}E_9$, and 0.1 mM PMSF, pH 11, neutralized with 50 mM Tris–HCl, pH 8.4, followed by buffer exchange for PBS containing either NP-40 or $C_{12}E_9$.

Peptide Dissociation Assay. The dissociation rates of biotinylated peptides from DR3 were measured essentially as described (24, 25) using affinity-purified HLA-DR3 molecules (25, 26). In brief, DR3 complexes were preloaded with 2 μM biotinylated peptides for 3 d (37°C, pH 4.5) in binding buffer containing 0.1% NP-40 or $C_{12}E_9$, pH 4.5. Excess peptides were removed by 10-K ultrafiltration (Amicon Corp.). The time course of peptide dissociation from the preloaded DR3 complexes was determined during 48 h at 37°C in binding buffer using 10 nM of DR3 complexes in the presence or absence of 50 nM sDM and an excess of 50 μM unlabeled ApoB(2877–2894) (Table 1). MHC–peptide complexes were immunoprecipitated with immobilized L243 and peptides were detected via streptavidin and biotinylated peroxidase (24, 25). The absorbance at 405 nm was measured by an ELISA reader (Multiskan Plus, Titertek, Meckenheim, Germany) and nonspecific signals (quadruplicates, typically 15% of maximal absorbance) were subtracted from the data.

SDS-PAGE, Native PAGE, and Western Blot Analysis. SDS-PAGE of purified sDM was performed on 12.5% polyacrylamide gels after boiling of the samples for 5 min in reducing Laemmli sample buffer. For native PAGE analysis, samples were incubated in non-reducing sample buffer for 30 min at room temperature. Western blot analyses were performed as described (21).

Results and Discussion

To study the function of HLA-DM independent from any associated proteins, recombinant soluble DM (sDM) was generated using a baculovirus expression system. The recombinant material was purified via a His-tag attached to the DMβ chain, using metal chelate affinity chromatography. The purity and composition of the isolated complex was assessed by SDS-PAGE and Western blot analyses. The isolated product was highly pure in that it consisted solely of two proteins with apparent molecular masses of 28kD and 29kD (Fig. 1, *C* and *B*) that were confirmed to be DMα and DMβ chains, respectively, using DMα and DMβ-His-tag–specific mAbs (Fig. 1, α and β). Native PAGE demonstrated that the sDM was secreted as a heterodimeric

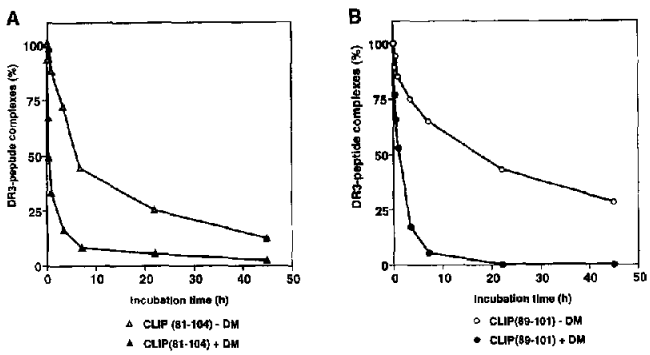

Figure 2. Ability of sDM to catalyze peptide dissociation from CLIP–DR3 complexes containing or lacking the NH_2-terminal fragment of CLIP. The timecourse of in vitro dissociation of biotinylated peptides bound to isolated DR3 was measured in the presence or absence of sDM. Data shown are derived from a representative set of values of five individual experiments. DR3 molecules were preloaded with CLIP(81–104) (*A*) or with CLIP(89–101) (*B*) (Table 1). Exchange of NP-40 for $C_{12}E_9$ in the assays did not change the outcome of the experiments, indicating that the findings were not due to the use of 0.1% NP-40 in the assay.

complex, recognized by both the DMα and DMβ antibodies, without any detectable aggregation.

The efficacy of sDM was tested by studying its catalytic effect on peptide exchange on DR3 in an in vitro peptide dissociation assay using DR molecules isolated from the DM-negative cell line T2.DR3 (8). In this cell line, DR3 is almost exclusively associated with CLIP facilitating loading of exogenous peptides onto the DR molecules because of the high spontaneous disssociation rates of endogenously bound CLIP. Recombinant sDM catalyzed the dissociation of CLIP from CLIP–DR3 complexes (Fig. 2 *A*), confirming the results of Sloan et al. (19). The catalytic effect could still be measured when sDM was applied in substoichiometric amounts compared with DR (1:3 ratio) and with an pH optimum for catalysis ranging between 4.5–5.5 (data not shown).

It is possible that the CLIP–DR complex is a preferential substrate for DM because of specific features of CLIP. The NH_2-terminal extension of the CLIP peptide extending from the peptide-binding groove of DR (residues 81–89) has been implicated in self-release of CLIP from DR (27, 28). To investigate whether this extension helped peptide release by DM we preloaded DR3 molecules with either CLIP(81–104) or CLIP(89–101), lacking the extension but containing the complete groove binding region (Table 1), and studied the dissociation rates of these peptides from DR3 with or without DM. In the absence of DM the dissociation of CLIP(81–104) was faster than that of CLIP(89–101) ($t_{1/2}$ ≈6.25 and 18 h, respectively). The catalytic function of DM was indiscriminate and dissociation rates for both peptides were dramatically increased ($t_{1/2}$ ≈0.16 and 1.25 h, respectively) (Fig 2, *A* and *B*). Thus, in this assay, the general mechanism underlying the catalytic action of DM on CLIP dissociation is not dependent on the NH_2-terminal extension of CLIP. Moreover, DM-enhanced release of CLIP(81–104) was independent of the presence of excess CLIP(81–92) added in trans (data not shown), suggesting that DM does not interact with CLIP(81–92) to exert its catalytic function. Taken together with the crystallographic data on the CLIP–DR complex (7), our findings imply that there are no obvious features of the CLIP–DR complex to single it out as a substrate for DM in the cellular pool of peptide–class II complexes.

To determine why only some peptide–MHC complexes are substrates for DM(17), we considered which other factors could play a role in the process of DM-mediated peptide dissociation from DR. The interaction between peptides and class II molecules is determined by the DR groove and the primary sequence of the peptide. For natural ligands, the core of the peptide contains allele-specific contact sites that are used as anchoring residues to the class II groove. Conventional antigens found associated with HLA-DR3 typically contain a hydrophobic/aromatic amino acid three to four residues from the NH_2 terminus (at relative position 1; P1), a similar residue at P9, an aspartate forming the DR3-specific contact site at P4, and a polar auxiliary anchor at P6. All of these residues anchor in complementary pockets in DR3 (23, 25). In the case of CLIP, the DR-binding motif is designed for promiscuous binding to different class II alleles via hydrophobic residues at P1 and P9. No allele-specific contact sites are present, since these could jeopardize binding to other class II molecules (7, 29). The DM sensitivity of the αβCLIP complex may be a reflection of this difference in binding motif and thus DM-mediated peptide release might depend on the degree of optimal interactions between peptide and DR-binding groove.

To test this hypothesis, peptide dissociation studies were performed using a range of CLIP variants and antigenic peptides together with derivatives modulated at DR3 anchoring positions (Table 1). Introduction of the DR3-specific anchor Asp in CLIP(81–104) at P4 led to a stable complex between peptide and DR ($t_{1/2}$ >>>48 h) that was no longer sensitive to release by DM (Fig. 3 *A*). Substitution of Pro_{112} at P6 for the anchoring residue Lys in CLIP(89–101), containing the minimal groove binding region, resulted only in a minor increase in stability of the peptide-DR complex ($t_{1/2}$ ≈22 h) (Fig. 3 *B*). Addition of DM still led to a release of the peptide ($t_{1/2}$ ≈3 h) (Fig. 3 *B*), but only half as efficiently as the unsubstituted CLIP(89–101) (Fig. 4). Introduction of anchoring residues at both P4 and P6 generated a stable CLIP–DR complex, and now DM was no longer able to induce dissociation of the peptide (see Fig. 3 *B*). Conversely, substitution of the DR3-specific anchor Asp_{2884} for Ala at P4 in the natural ligand ApoB(2877–2894) (Table 1), enhancing spontaneous dissociation of the peptide from the DR complex, made the previously insensitive complex highly vulnerable to the catalytic action of DM (see Fig. 3 *C*). The HAChRα(312–325) and MOMP(251-265) peptides bind DR3 with a high binding capacity (6, 19, 25), but contain suboptimal anchoring residues in some positions with HAChRα(312–325) containing the least optimal binding motif (Table 1). The spontaneous dissociation rate of both peptides from

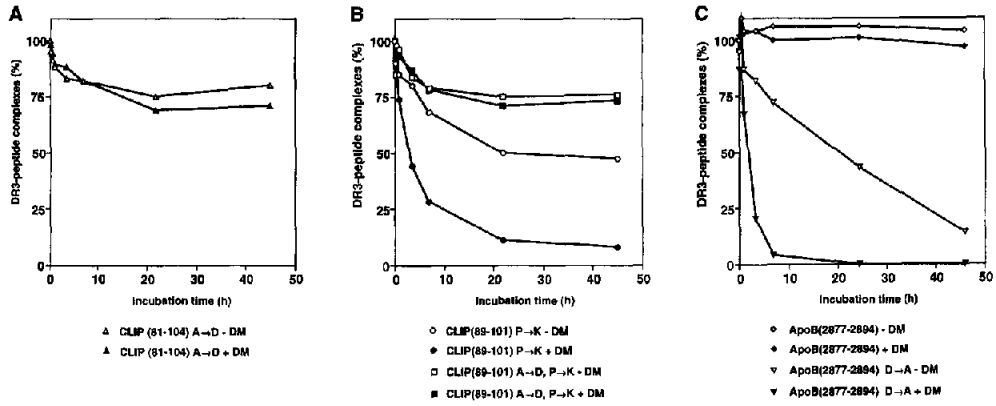

Figure 3. Ability of sDM to catalyze peptide dissociation from peptide–DR3 complexes depends on the primary sequence of the peptide complexes to DR3. The timecourse of dissociation of CLIP(81–104) containing a DR3-specific anchor at P4 from DR3 was measured in the presence or absence of sDM (*A*). Effect of introduction of one or two anchoring residues in CLIP(89–101) CLIP(89–101) P→K and CLIP(89–101) A→D, P→K, respectively, Table 1) on sDM-catalyzed peptide dissociation from DR3 (*B*) Dissociation of ApoB (2877–2894) with and without the DR3-specific anchor Asp at P4 (Table 1) from isolated DR3 molecules in the presence or absence of sDM (*C*). All figures shown are derived from one representative experiment out of five individual experiments.

DR3 was low, but faster for HAChRα(312–325) than for MOMP(251–265) (Fig. 4). The dissociation rate of HAChRα(312–325) from DR3 was sensitive to DM catalysis, whereas the more stable MOMP–DR3 complex was mostly resistent to the action of DM (Fig. 4). Since the HAChRα(312–325) peptide contains an Asp at P4, but has suboptimal anchors for the other contact sites, and MOMP (251–265) lacks the Asp, but otherwise contains an optimal binding motif, these peptides show that the DM sensitivity of peptide–DR3 complexes was not solely dependent on the absence or presence of the DR3-specific anchor. Taken together, the data demonstrate that the susceptibility of the peptide–DR3 complex for DM correlated with the overall number of positive interactions anchoring the peptide to the peptide binding groove of DR (Fig. 4; Table 1).

In conclusion, the data presented here imply that DM functions as a general catalyst to dissociate peptides bound to DR3. These data are consistent with the observation of a transient interaction between DM and DR, irrespective of loading with cognate peptide or CLIP (21). The primary sequence of the peptide itself seems to be the factor determining whether it can be released by DM. The complement of optimal anchoring residues in the peptide dictates the susceptibility of the complex for DM-mediated peptide release, probably by influencing the stability and free energy of the peptide–DR3 complex. The composition of the nonanchoring residues in the peptide core, together with the regions flanking the core, may contribute to this process in a more subtle manner.

The consequence of the unrestricted action of DM is that in vivo all peptides bound to DR are submitted to the action of DM. Only the most stably bound peptides, presumably selected by a stochastic process, will remain associated with the class II molecule to be exported to the cell surface for antigen presentation. Thus, DM has an editor function (17), optimizing presentation of appropriate peptides. One could imagine that peptides containing less optimal anchors also reach the cell surface on DR when present in high amounts or by having an intermediate off-rate. A role of DM may be to reduce presentation of these

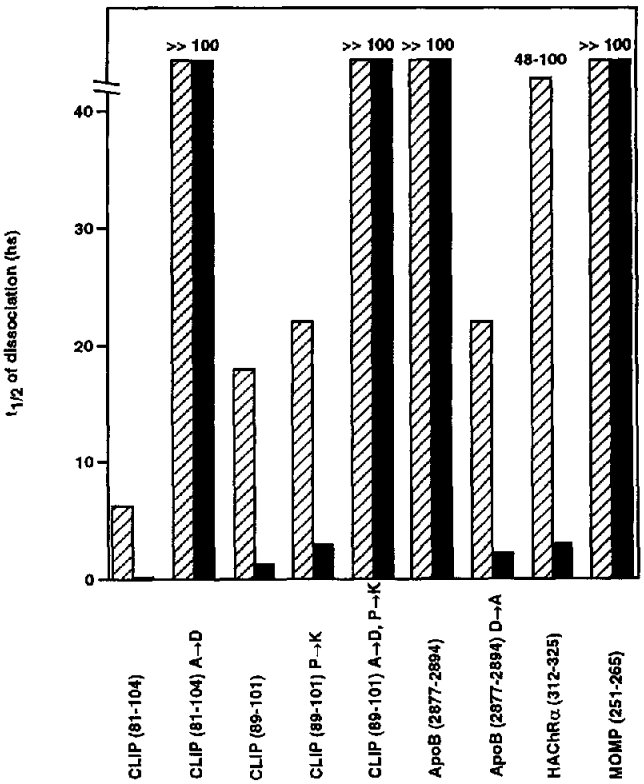

Figure 4. Effect of sDM on dissociation rates of CLIP variants and antigenic peptides, either modulated or not at DR3 anchoring positions, from isolated DR3 complexes. $t_{1/2}$ values of dissociation of prebound peptides to DR3 molecules were determined from the timecourse of dissociation of peptide from DR3 and represent a mean value of five individual experiments. Dissociation was measured in the absence (*hatched bars*) or presence (*solid bars*) of sDM. In cases in which a 50% dissociation of peptide from DR3 was not reached within 48 h $t_{1/2}$ values were are divided into two groups denoted as $48 < t_{1/2} < 100$ h, and as $>> 100$ h

peptides as far as possible. In this scheme, the level of DM and DR in MIIC vesicles of different tissues could be of relevance for autoimmune diseases that may depend on presentation of self-peptides with suboptimal anchors (30).

We thank P. Marrack for generously providing pBACp10p; P. Cresswell for the T2-DR3 cells; A. Coffer, G. Chalkley, and J. Fellow for excellent technical advice; and our colleagues, especially N. Koch, C. Thomas, C. Roucard, and F. Sanderson for helpful suggestions and reagents.

This work was supported by an institutional fellowship from the European Community (ERB-CHBG-CT93-0463) for S.M. van Ham, by Boehringer Ingelheim Fonds for U. Grüneberg, and by SFB120 for G. Malcherek and A. Melms.

Address correspondence to John Trowsdale, Human Immunogenetics Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom.

Received for publication 15 July 1996 and in revised form 9 September 1996.

References

1. Guagliardi, L.E., B. Koppelman, J. Blum, M.S. Marks, P. Creswell, and F.M. Brodsky. 1990. Co-localisation of molecules involved in antigen processing and presentation in an early endocytic compartment. *Nature (Lond.).* 343:133–139.
2. Neefjes, J.J., V. Stollorz, P.J. Peters, H.J. Geuze, and H.L. Ploegh. 1990. The biosynthetic pathway of MHC class II but not class I molecules intersects the endocytic route. *Cell.* 61: 171–183.
3. Roche, P.A., and P. Cresswell. 1990. Invariant chain association with DR molecules inhibits immunogenic peptide binding. *Nature (Lond.).* 345:615–618.
4. Blum, J.S., and P. Cresswell. 1988. Role of intracellular proteases in the processing and transport of class II HLA antigens. *Proc. Natl. Acad. Sci. USA.* 85:3975–3979.
5. Peters, P.J., J.J. Neefjes, V. Oorschot, H.L. Ploegh, and H.J. Geuze. 1991. Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosmal compartments. *Nature (Lond.).* 349:669–676.
6. Avva, R.R., and P. Cresswell. 1994. In vivo and in vitro formation and dissociation of HLA-DR complexes with invariant chain–derived peptides. *Immunity.* 1:763–774.
7. Ghosh, P., M. Amaya, E. Mellins, and D.C. Wiley. 1995. The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. *Nature (Lond.).* 378:457–462.
8. Riberdy, J.M., J.R. Newcomb, M.J. Surnam, 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.
9. Miyazaki, T., P. Wolf, S. Tourne, C. Waltzinger, A. Dierich, N. Barois, H. Ploegh, C. Benoist, and D. Mathis. 1996. Mice lacking H2-M complexes, enigmatic elements of the MHC class II peptide-loading pathway. *Cell.* 84:531–541.
10. Martin, W.D., G.G. Hicks, S.K. Mendiratta, H.I. Leva, H.E. Ruley, and L. Van Kaer. 1996. H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell.* 84:543–550.
11. Denzin, L.K., N.F. Robbins, C. Carboy-Newcomb, and P. Cresswell. 1994. Assembly and intracellular transport of HLA-DM and correction of the class II antigen-processing defect in T2 cells. *Immunity.* 1:1–20.
12. 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.
13. Kelly, A.P., J.J. Monaco, S. Cho, and J. Trowsdale. 1991. A new human HLA class II–related locus, DM. *Nature (Lond.).* 353:571–573.
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. Karlsson, L., A. Peleraux, R. Lindstedt, M. Liljedahl, and P.A. Peterson. 1994. Reconstitution of an operational MHC class II compartment in nonantigen-presenting cells. *Science (Wash. DC).* 266:1569–1573.
16. Sanderson, F., M.J. Kleijmeer, A.P. Kelly, D. Verwoerd, A. Tulp, J.J. Neefjes, 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.
17. Sloan, V.S., P. Cameron, G. Porter, M. Gammon, M. Amaya, E. Mellins, and D.M. Zaller. 1995. Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature (Lond.).* 375:802–806.
18. 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.
19. Denzin, L.K., and P. Cresswell. 1995. HLA-DM induces CLIP dissociation from MHC class II alpha beta dimers and facilitates peptide loading. *Cell.* 82:155–165.
20. Riberdy, J.M., 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.
21. Sanderson, F., C. Thomas, J. Neefjes, and J. Trowsdale. 1996. Association between HLA-DM and HLA-DR in vivo. *Immunity.* 4:1–20.
22. Kozono, H., J. White, J. Clements, P. Marrack, and J. Kappler. 1994. Production of soluble MHC class II proteins with covalently bound single peptides. *Nature (Lond.).* 369:151–154.
23. Malcherek, G., K. Falk, O. Roetzschke, H.-G. Rammensee, S. Stevanovic, V. Gnau, G. Jung, and A. Melms. 1993. Natural peptide ligand motifs of two HLA molecules associated with myasthenia gravis. *Int. Immunol.* 5:1229–1237.
24. Jensen, P.E. 1992. Long-lived complexes between peptide and major histocompatibility complexes are formed at low pH with no requirement for pH neutralization. *J. Exp. Med.* 176:793–798.
25. Malcherek, G., V. Gnau, S. Stevanovic, H.-G. Rammensee, G. Jung, and A. Melms. 1994. Analysis of allele-specific contact sites of natural HLA-DR17 ligands. *J. Immunol.* 153:

1141–1149.
26. Lampson, L.A., and R. Levy. 1980. Two populations of Ia-like molecules on a human cell line. *J. Immunol.* 125:293–299.
27. Urban, R.G., R.M. Chicz, and J.L. Strominger. 1994. Selective release of some invariant chain–derived peptides from HLA-DR1 molecules at endosomal pH. *J. Exp. Med.* 180: 751–755.
28. Kropshofer, J., A.B. Vogt, L.J. Stern, and G.J. Hämmerling. 1995. Self-release of CLIP in peptide loading of HLA-DR molecules. *Science (Wash. DC).* 270:1357–1359.
29. Malcherek, G., V. Gnau, G. Jung, H.-G. Rammensee, and A. Melms. 1995. Supermotifs enable natural invariant chain–derived peptides to interact with many major histocompatibility complex–class II molecules. *J. Exp. Med.* 181:527.
30. Mason, K., D.J. Denney, and H.M. McConnell. 1995. Myelin basic protein peptide complexes with the class II MHC molecules I-A(U) and I-A(K) form and dissociate rapidly at neutral pH. *J. Immunol.* 154:5216–5227.