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, Universität Tübingen, 72076 Tübingen, 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 (PharMmgen, 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.
Table 1. Alignment of Peptides According to the HLA-DR3 Binding Motif

| Antigen   | Sequence                          | Relative position |
|-----------|-----------------------------------|-------------------|
| CLIP (81-104) | LPKPEFKPSKRMATFLLMQALPM          | 1                 |
| CLIP (81-104) A→D | LPKPKPSKRMATFLLMQALPM          | 4                 |
| CLIP (89-101) P→K | SKRMATFLLMQALPM          | 8                 |
| CLIP (89-101) A→D, P→K | SKRMATFLLMQALPM          | 12                |
| CLIP (81-92) | LPKPEFKPSKMR            | 16                |
| ApoB (2877-2894) | ISNQLTDNIKFHKLN       | 21                |
| ApoB (2877-2894) D→A | ISNQLTDNIKFHKLN       | 25                |
| HACHhα (312-325) | VKRVLPSSTPSQ     | 30                |
| MOMP (251-265) | QASLALSYRLMNMTF   | 35                |

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 SC1 (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 SC1 for the DMα chain (lane α) or the DMβ-specific antiserum FS4 (lane β).

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 complex.
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 endogenous peptides onto the DR molecules because of the high spontaneous dissociation 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 stoichiometric 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 NH2-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). The catalytic function of DM was not detectable for peptides that were preloaded with CLIP(81-89) or CLIP(89-101) (Fig. 2 A). Exchange of NP-40 for CI2E9 in the assay 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.
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(89–104) 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(89–104) 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 HACtR(312–325) than for MOMP(251–265) (Fig. 4). The dissociation rate of HACtR(312–325) from DR3 was sensitive to DM catalysis, whereas the more stable MOMP–DR3 complex was mostly resistant to the action of DM (Fig. 4). Since the HACtR(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 as far as possible. In this scheme, the level of DM and DR in MIIIC vesicles of different tissues could be of relevance for autoimmune diseases that may depend on presentation of self-peptides with suboptimal anchors (30).
References

1. Guagliardl, L.E., B. Koppelman, J. Blum, M.S. Marks, P. Cresswell, 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 processes 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. Oursouch, 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 lysosomal 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. Ghoosh, P., M. Anaya, E. Mellins, and D.C. Wiley. 1995. The structure of an intermediate in class II MHC maturation: CLIP binding to HLA-DR3. Nature (Lond.). 378:457-462.

8. Ribefry, 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. Diener, N. Barais, 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. Mendrattta, H.L. 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, I.K., N.F. Robhms, 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. Flung, S.P., B. Arp, and D. Paus. 1994. HLA-DM and -DBM genes are both required for MHC class II/peptide complex formation in antigen-presenting cells. Nature (Lond.). 369: 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. Shanan, 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. Pelaex, R. Lindstedt, M. Liljedahl, and P.A. Peterson. 1994. Reconstitution of an operational MHC class II compartment in nonantigen-presenting cells. Science (Wash. DC). 266:1566-1569.

16. Sanderson, F., M.J. Kleiinmeen, 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. Slomn, 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, I.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. Ribery, 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. Ramannsee, S. Stevanovic, V. Gna, G. Jug, 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. Gna, S. Stevanovic, H.-G. Ramannsee, G. Jug, and A. Melms. 1994. Analysis of allele-specific contact sites of natural HLA-DR17 ligands. J. Immunol. 153:2023. van Ham et al. Brief Definitive Report
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. Giard, 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.