Specialized Functions of Major Histocompatibility Complex Class I Molecules. II. Hmt Binds N-Formylated Peptides of Mitochondrial and Prokaryotic Origin

By Said M. Shawar,[*][‡] Jatin M. Vyas,[‡] John R. Rodgers,[‡]
Richard G. Cook,[‡] and Robert R. Rich[*][‡][§]

From the *Howard Hughes Medical Institute Laboratory, the ‡Department of Microbiology and Immunology, and the §Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

Summary

The physiological functions of the mouse telomeric major histocompatibility complex (MHC) class I molecules, including Hmt, are unknown. Hmt presents a polymorphic, N-formylated peptide encoded by the mitochondrial gene ND1 forming the cell surface maternally transmitted antigen (Mta). Because the N-formyl moiety is required for Hmt binding, we proposed that Hmt may function generally in presentation of N-formylated antigens. This hypothesis was validated by a competitive binding assay, demonstrating that synthetic N-formyl peptides from other mitochondrial genes also bound Hmt. Bacteria similarly initiate protein synthesis with N-formylmethionine; indeed, we established that Hmt can also present prokaryotic peptides in an N-formyl–dependent manner. These results indicate biochemical specialization of this MHC-peptide interaction and suggest a unique role for Hmt in prokaryotic host defenses.

Mouse MHC class I genes are classified into two groups, the polymorphic H-2 K, D, and L genes, located in the centromeric segment of the H-2 complex (1, 2), and the more numerous and less polymorphic "nonclassical" telomeric genes encoded in the H-2 Q, T, and M (formerly Qa-Tla-Hmt) regions (2). While products of the former are known to bind and present antigens to CD8^{+} T cells (3), the function(s) of the latter genes are unknown (4). These nonclassical MHC class I genes could provide a repository of genetic diversity available to the H-2 K, D, and L regions through gene conversion or could be evolutionary remnants devoid of function (1, 3). Alternatively, their products could play a role in differentiation, immune regulation, or antigen presentation (5). Computer-aided modeling suggested that the structure of the telomeric class I molecules resembles that of HLA-A2, including a possible peptide binding site (6). Two such molecules, Qa-1 (7) and Hmt (8–10), can present synthetic peptides to T cells, and several nonclassical MHC molecules can stimulate alloreactive MHC class I response (11, 12). However, these observations did not suggest a specific physiological role for the nonclassical MHC class I molecules.

We previously demonstrated that the product of one of these genes, Hmt, presents an N-formylated, mitochondrially encoded peptide (designated Mtf) to antigen-specific CTL, thereby forming the maternally transmitted antigen (Mta) (8). Mtf itself derives from the NH_2 terminus of the mitochondrially encoded NADH dehydrogenase subunit 1

(ND1) (8–10). The alleles of Mtf (α, β, γ, and δ) that account for the polymorphism of Mta (designated a, b, c, and d) differ at codon 6 of *ND1* (10). The most common allelic product, Mtf$^\alpha$, contains isoleucine in position six, while Mtf$^\beta$ contains alanine. When bound to Hmt and β_2-microglobulin these two peptides are recognized by Mta^a- and Mta^b-specific CTLs, respectively. Moreover, the exogenous addition of synthetic Mtf$^\alpha$ to cells expressing Hmt and endogenous Mtf$^\beta$ sensitizes them to lysis by Mta^a-specific CTLs (8, 9).

The exclusive presentation of Mtf by Hmt and the failure to detect other examples of Hmt-restriction suggested that unique biochemical properties of mitochondrially encoded peptides might favor presentation by Hmt (8, 13). Most obviously, mitochondrial, but not cytoplasmic, protein synthesis is initiated with N-formylmethionine (14). Indeed, N-formylated ND1 synthetic peptides, but not N-acetylated or nonsubstituted analogs, were shown to bind to Hmt (8, 9). Inasmuch as bacteria also initiate protein synthesis with N-formylmethionine, it further seemed plausible that Hmt might function in selective presentation of N-formyl-peptides derived from prokaryotic organisms.

Materials and Methods

Peptide Synthesis. Peptides in Table 1 were synthesized by solid-phase on a peptide synthesizer (430A; Applied Biosystems, Foster

City, CA). As described previously (8) peptides were assayed for purity by reverse-phase HPLC and amino acid analysis (Pico Tag system; Waters Associates, Milford, MA); several peptides were also analyzed by NH_2-terminal amino acid sequence analysis (477A Protein Sequencing System; Applied Biosystems).

Cytotoxic T Lymphocytes. CTL clone 1D3 specific for Mta[a] was generated as described (8) by immunizing Mta[b] (NZB ♀ × BALB/c ♂) F_1 mice with BALB/c (Mta[a]) spleen cells. Restimulation in vitro and maintenance were as described earlier (8).

Competition Assay. Target cells of the WEHI-105.7 NZB thymoma (H-2[d], Mta[b]) were labeled with ^{51}Cr, washed four times, and incubated for 75 min at 37°C with increasing concentrations of the indicated peptides in the presence of 50 nM $fND1_{1-12}$. Target cells were washed twice before incubation with Mta[a]-specific CTL clone 1D3 in a 4-h ^{51}Cr release assay, using an effector to target (E/T) cell ratio of 20:1. Percent specific lysis was calculated as described previously (8).

Results and Discussion

To examine binding of diverse peptides to Hmt we established a peptide competition assay. Whereas a six amino acid formyl peptide derived from ND1 ($fND1_{1-6}$; Table 1) defined the minimum sequence for sensitization of target cells for Mta-specific lysis (8), a formylated five amino acid ND1 peptide ($fND1_{1-5}$) efficiently competed for Hmt binding. Consequently, $fND1_{1-5}$ blocked target cell sensitization (Fig. 1) by such sensitizing peptides as $fND1_{1-12}$. The competition by $fND1_{1-5}$ could be reversed by increasing concentrations of antigenic peptide. This pattern of inhibition was seen with all Mta-specific CTLs tested. Neither nonsubstituted nor N-acetylated $NB1_{1-5}$ blocked target cell lysis (Table 1), confirming the N-formyl requirement for Hmt binding. These results argue for competitive occupancy of a single binding site by both sensitizing and blocking peptides.

To test the hypothesis that Hmt could bind NH_2-terminal bacterial peptides, we first mediated ND1 peptides with a positively-charged lysine residue to simulate typical prokaryotic signal peptides (15). Despite the introduction of this charged residue, such peptides also efficiently blocked Mta-specific cytolysis (Fig. 2, *A*). To evaluate whether an N-formyl group was a general requirement for Hmt-peptide interactions or only reflected the peculiarities of ND1 and its analogs, we examined whether other synthetic mitochondrial peptides could block $fND1_{1-12}$ induced lysis. One such peptide, $fND2_{1-12}$ (Fig. 2 *B*), did not inhibit. In contrast, $fND4_{1-12}$ and $fND5_{1-12}$ peptides efficiently competed for binding of

Table 1. *Synthetic Peptides Used in Competition Assays*

Peptide designation	Sequence	IC_{50}
		μM[*]
Mitochondrial peptides		
$ND1_{1-5}$[†]	MFFIN	<0.2
$ND1_{1-6}$	MFFINI	NA[§]
$ND1_{1-12}$	MFFINILTLLVP	NA
$ND1_{1-12/K2}$	MKFINILTLLVP	<2
$ND1_{1-12/K3}$	MFKINILTLLVP	<2
$ND2_{1-12}$	MNPITLAIIYFT	>20
$ND4_{1-12}$	MLKIILPSLMLL	<3
$ND5_{1-12}$	MKVINIFTTSIL	<2
Nonmitochondrial peptides		
E. coli amp-C β-Lactamase (Amp-C)[‖]	MFKTTLCALLIT	<6
B. cereus bla-z β-Lactamase (Bla-z)	MFVLNKFF	<0.1
E. coli ribosomal protein L25 (L25)	MFTINAEV	<3
V. harveyi alkanal monooxygenase (VHAM)	MKFGNFLL	>20
V. anguillarum membrane associated protein (VAMAP)	MFKSTLNIAV	>20
C. nephridii thioredoxin C-2 (CNTC)	MMFKFALYFI	>20

[*] IC_{50}: The formyl peptide concentration required for 50% inhibition of target lysis of cells sensitized with 50 nM of $fND1_{1-12}$. Calculated IC_{50} are representative of three or more independent experiments. The nonsubstituted and N-acetylated analogs of all peptides listed except $ND2_{1-12}$ were also tested and the IC_{50} of each was >20 μM.
[†] Convention for mitochondrial peptides: $ND1_{1-5}$ designates a nonsubstituted pentameric peptide of ND1 spanning residues 1 to 5; $fND1_{1-5}$ and $AcND1_{1-5}$ are formylated and acetylated derivatives, respectively. $ND1_{1-12/K2}$ is a 12-mer ND1 peptide modified by substitution of lysine in position 2. $ND2_{1-12}$ denotes the NH_2-terminal 12-mer peptide of the NADH dehydrogenase subunit 2, etc.
[§] NA: not applicable. Sensitizing peptides.
[‖] Designations of nonmitochondrial peptides. These sequence data are from the GenBank database; accession numbers of the precursor proteins are: Amp-C:A01007; Bla-Z:A27755; L25:R5EC25; VAHM:A22613; VAMAP:A29928; and CNTC:A29797.
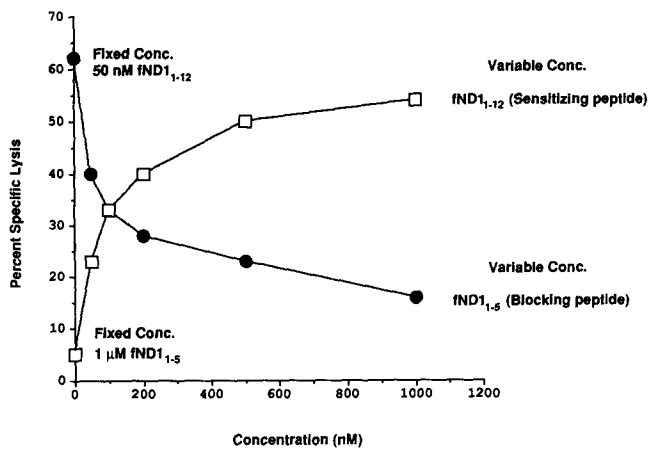
Figure 1. Competitive binding of synthetic peptides to Hmt. ^{51}Cr-labeled target cells (WEHI-105.7) were incubated for 75 min at 37°C with increasing concentrations of fND1$_{1-12}$ in the presence of 1 μM fND1$_{1-5}$ ($\square$), or increasing concentrations of fND1$_{1-5}$ in the presence of 50 nM fND1$_{1-12}$ ($\bullet$). Target cells were washed twice before incubation with Mta^a-specific CTL clone 1D3 in a 4-h ^{51}Cr release assay, using an effector to target (E/T) cell ratio of 20:1.

fND1$_{1-12}$ to Hmt with an IC$_{50}$ of 1–3 μM (Fig. 2, C, and D). Significantly, neither acetylated nor unmodified forms of ND4$_{1-12}$ or ND5$_{1-12}$ peptides blocked Mta-specific cytolysis. Preliminary experiments indicate that substitution of phenylalanine for proline in position 3 of fND2$_{1-12}$ similarly converts this peptide to an effective competitor.

Finally, we examined whether Hmt could bind naturally occurring non-mitochondrial peptides in an N-formyl depen-
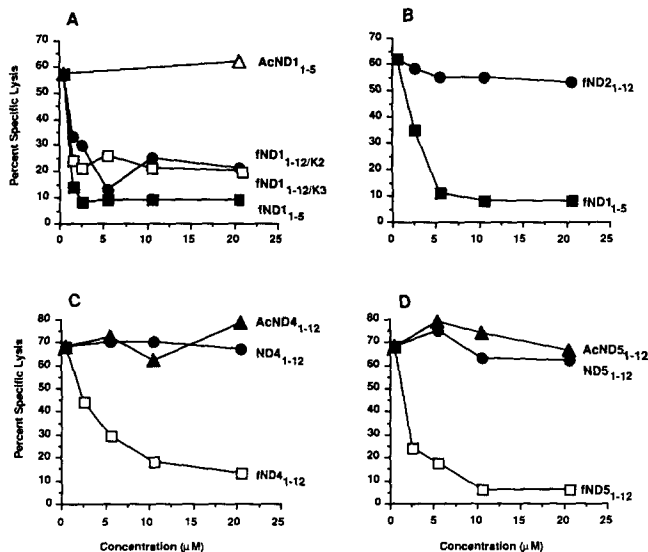
Figure 2. ND1 analogs and mitochondrially-encoded peptides other than ND1 bind to Hmt. Increasing concentrations of the indicated peptides were added with 50 nM fND1$_{1-12}$ to ^{51}Cr-labeled WEHI-105.7 target cells for 75 min. Target cells were incubated with Mta^a-specific clone 1D3 at a 20:1 E/T ratio. (A) ND1$_{1-12}$/K2 and ND1$_{1-12}$/K3. (B) ND2$_{1-12}$. (C) ND4$_{1-12}$. (D) ND5$_{1-12}$.
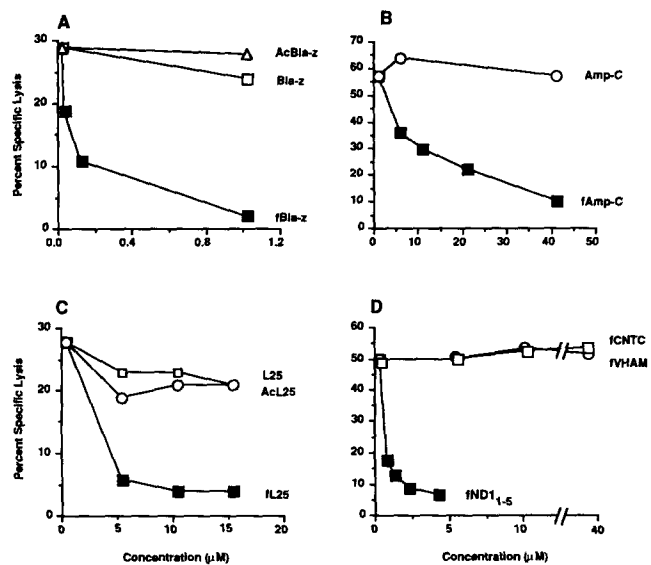
Figure 3. Prokaryotic N-formylated peptides compete for binding to Hmt. ^{51}Cr-release competition assay was performed as described in Fig. 2. Peptide-pulsed WEHI 105.7 target cells (Mta^b) were incubated with Mta^a-specific CTL clone 1D3 at an E/T ratio of 20:1. fND1$_{1-5}$ and AcND1$_{1-5}$ were used as positive and negative controls, respectively. (A) Bla-z. (B) Amp-C. (C) L25. (D) CNTC and VHAM.

dent fashion. Six synthetic peptides were chosen based on partial sequence similarity (25–50% including the first position) to ND1, ND4 or ND5 peptides. NH$_2$-terminal peptides of *Escherichia coli* amp-C β-lactamase, *E. coli* ribosomal protein L25 and *Bacillus cereus* bla-z β-lactamase (Table 1) blocked Mta^a-specific target cell lysis, exhibiting N-formyl dependent competitive binding to Hmt (Fig. 3). Thus, Hmt binds a variety of formyl peptides including certain bacterial signal sequences.

The N-formyl group may be necessary, but it is not sufficient for Hmt binding; three other N-formylated prokaryotic peptides did not block Mta-specific lysis. The signal sequences from *Corynebacterium nephridii* thioredoxin C-2 (CNTC), *Vibrio anguillarum* membrane associated protein (VAMAP) and *V. harveyi* alkanal monooxygenase (VHAM) (Table 1), did not block Mta^a-specific target cell lysis (Fig. 3 and Table 1). Thus, as expected, we conclude that the binding cleft of Hmt contains important contact points other than those interacting with the formyl group. Although the Hmt-binding peptides in this study have no apparent motif or secondary structure, such might be inferred after a larger number of binding peptides has been investigated (9).

Hmt appears not to accommodate the extra methyl group of the N-acetyl moiety, suggesting a tight formyl-peptide binding pocket within the peptide-binding cleft of Hmt. These results are thus consistent with suggestions by Schumacher et al. (16) that peptide termini are tightly associated with the cleft of class I molecules. This pocket in Hmt seems to be evolutionarily conserved: Mta-specific CTLs reactive with the *Mus musculus domesticus* Mtf$^\alpha$ peptide are capable of lysing cells derived from murid species as distantly related as *M. caroli* and *M. dunni* ($\sim$16–20 million yr divergence time)

(13). Thus it is likely that both the peptide-binding cleft and the T cell receptor interface of Hmt have maintained critical structures required for antigen-presentation to T cells. Mta^a-specific CTL, like typical H-2KDL-restricted CTL, express α/β (9, 17) (TCR), rather than the γ/δ TCR that have been associated with some CTL responses restricted by telomeric H-2 molecules (7, 11, 12). It remains to be established whether the V_α or V_β segments used by the receptors are diverse or limited.

The immune system must balance the need to present foreign antigen with the need to avoid autoimmunity. Through a binding preference for N-formyl peptides, Hmt may employ an immunological strategy distinct from the generalist strategy of the H-2 KDL antigens. By focusing on N-formyl termini, Hmt may selectively present peptides derived from prokaryotic parasites (8, 13) while ignoring the vast majority of self antigens; this leaves only the autoimmune response to mitochondrial antigens to be handled by clonal deletion or suppression. As prokaryotic N-formylated peptides associate in vitro with Hmt, it may be possible to detect an Hmt-restricted CTL response by mice immunized with such peptides or infected with intracellular pathogens. Alternatively, but not exclusively, Hmt may subserve important nonimmunological functions. These could include transport from the cytoplasm to the exterior of the cell of hydrophobic formylated mitochondrial signal peptides, cleaved after insertion through the mitochondrial membrane.

Other nonclassical MHC molecules may, like Hmt, display a greater specialization of peptide binding than do the classical MHC molecules. As stable surface expression of class I MHC molecules appears to require peptide binding (9), selective binding could explain why the telomeric MHC molecules are poorly represented on the cell surface. Such specialization could similarly explain the limited polymorphism of nonclassical MHC molecules.

References

1. Klein, J., and F. Figueroa. 1986. Evolution of the major histocompatibility complex. Crit. Rev. Immunol. 6:265.
2. Klein, J., C. Benoist, C.S. David, P. Demant, K. Fischer Lindahl, L. Flaherty, R. Flavell, U. Hammerling, L. Hood, S. Hunt, III, P. Jones, P. Kourilsky, H. McDevitt, D. Meruelo, D. Murphy, S. Nathenson, D. Sachs, M. Steinmetz, S. Tonegawa, E. Wakeland, and E. Weiss. 1990. Revised nomenclature of mouse H-2 genes. Immunogenetics. 32:147.
3. Lawlor, D.A., J. Zemmour, P.D. Ennis, and P. Parham. 1990. Evolution of class-I MHC genes: From natural selection to thymic selection. Annu. Rev. Immunol. 8:23.
4. Stroynwoski, I. 1990. Molecules related to class-I major histocompatibility complex antigens. Annu. Rev. Immunol. 8:501.
5. Flaherty, L., E. Elliot, J. Tine, A. Walsh, and J. Waters. 1990. Immunogenetics of the Q and TL regions of the mouse. Crit. Rev. Immunol. 10:131.
6. Prochnicka-Chalufour, A., J. Casanova, P. Kourilsky, and J. Caverie. 1989. Extensive structural homology between H-2K/D/L antigens and non-polymorphic class I Qa, Tla and 37 molecules suggests they may act as possible carriers. Res. Immunol. 140:133.
7. Vidovic, D., M. Roglic, K. McKune, S. Guerder, C. MacKay, and Z. Dembic. 1989. Qa-1 restricted recognition of foreign antigen by a γ/δ T-cell hybridoma. Nature (Lond.). 340:646.
8. Shawar, S.M., R.G. Cook, J.R. Rodgers, and R.R. Rich. 1990. Specialized functions of MHC class I molecules. I. An N-formyl peptide receptor is required for construction of the class I antigen Mta. J. Exp. Med. 171:897.
9. Shawar, M., J.R. Rodgers, R. Cook, and R.R. Rich. 1991. Specialized function of the non-classical MHC class I molecule Hmt: a specific receptor for N-formylated peptides. Immunol. Res. In press.
10. Loveland, B., C. Wang, H. Yonekawa, E. Hermel, and K. Fischer Lindahl. 1990. Maternally transmitted histocompatibility antigen of mice: A hydrophobic peptide of a mitochondrially encoded protein. Cell. 60:971.
11. Kastner, D.L., and R.R. Rich. 1979. H-2 nonrestricted cytotoxic responses to an antigen encoded telomeric to H-2D. J. Immunol. 122:196.
12. Ito, K., L. Van Kaer, M. Bonneville, S. Hsu, D. Murphy, and S. Tonegawa. 1989. Recognition of the product of a novel MHC TL region gene (27^b) by a mouse γ/δ T cell receptor. Cell. 62:549.
13. Rodgers, J.R., R. Smith, III, M.W. Huston, and R.R. Rich. 1986. Maternally transmitted antigen. Adv. Immunol. 38:313.
14. Buetow, D., and W. Wood. 1978. The mitochondrial translation system. Subcell. Biochem. 5:1.
15. Duffaud, G., S. Lenhardt, P. March, and M. Inouye. 1985. Structure and function of the signal peptide. Curr. Top. Membranes Transport. 24:65.
16. Schumacher, T., M. De Bruijn, L. Vernie, W. Kast, G. Melief, J. Neefjes, and H. Ploegh. 1991. Peptide selection by MHC class I molecules. Nature (Lond.). 350:703.
17. Fisher Lindahl, K., E. Hermel, B. Loveland, S. Richards, and C. Wang. 1991. Maternally transmitted antigen of mice. A model transplantation antigen. Annu. Rev. Immunol. 9:351.