SPECIALIZED FUNCTIONS OF MHC CLASS I MOLECULES

I. An N-Formyl Peptide Receptor Is Required for Construction of the Class I Antigen Mta

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The MHC class I and II molecules play a central role in immune responses such as transplant rejection through their role in presentation of antigens to T lymphocytes (1, 2). Although the immunological functions of "classical" MHC molecules have been studied extensively (3), the biological functions of the class I molecules encoded telomeric to H-2D are unknown (4). Several hypotheses have been advanced to explain the expression of these nonclassical MHC-related molecules (3, 5). Thus, they may have functions distinct from antigen presentation, such as encoding receptors for macromolecules on cell surfaces or in solution, or they may simply be variants of the classical gene products, without qualitatively different function. Recently, Simister and Mostov (6) demonstrated that the Fc receptor (FcRn) that transports maternal IgG across the intestinal epithelium of newborn rats is an MHC class I-like molecule.

It is now widely accepted that class I and II MHC molecules bind fragments of protein antigens and present them to the antigen receptor of T lymphocytes (7-10). The use of synthetic peptides has facilitated the definition of minimum amino acid core sequences required for recognition by helper or cytotoxic T lymphocytes (11-13). Suggested structures of peptides functioning as T cell epitopes include an amphipathic α-helix with one surface interacting with the MHC molecule and another surface with the TCR (14, 15), or a linear pattern with a four or five amino acid motif core (16, 17).

The maternally transmitted antigen (Mta)1 is a murine cell surface MHC class I-like antigen defined exclusively by antigen-specific cell-mediated lympholysis (CML) (18-20). The expression of Mta is controlled by at least three genes, two of which are nuclear, i.e., β2-microglobulin (β2M) and Hmt, a locus telomeric to the T1a region of the H-2 complex (21). The third gene, which accounts for maternally inherited polymorphism, is mitochondrial (Mtf) (22-24). Four alleles of Mtf have been identified (α, β, γ, and δ), the most common of which, Mtfα, is found in >95% of inbred and wild mice (25, 26).

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1 Abbreviations used in this paper: CABs, Con A blasts; CML, cell-mediated lympholysis; Mta, maternally transmitted antigen; ND1, NADH dehydrogenase subunit 1.
Mta-ASSOCIATED N-FORMYL PEPTIDE RECEPTOR

Genetic analyses of monoplasmic and heteroplasmic hybrid cells showed that Mtf\textsuperscript{ex} and Mtf\textsuperscript{ax} were mitochondrial genes and could be expressed codominantly (24, 27). More recently, we showed that Mtf peptides were mitochondrially encoded and had unusually short half-lives (28); we suggested that Mtf encodes a signal peptide derived during maturation of respiratory enzymes. Indeed, Wang et al. (29) reported that a peptide from the NH\textsubscript{2} terminus of a 35-kD subunit of the mitochondrial NADH dehydrogenase (NDI) behaved in CML assays as an Mtf gene product. In addition, they found that the different Mtf alleles are created by substitution in codon 6 of NH1, which in the Mtf alleles α, β, γ, and δ encodes isoleucine, alanine, valine, and threonine, respectively.

Mta has the characteristics of an MHC class I molecule that functions in presentation of a self peptide at the surface of cells in some unique fashion. This phenomenon of self peptide presentation is seen on mouse cells of diverse tissue origin (24). The general phenomenon of binding and presentation of self peptides by MHC molecules was proposed more than 10 years ago (30) and was demonstrated more recently for class II molecules in vitro (31-33) and in vivo (34, 35). Lorenz and Allen (34) suggested that constitutive processing and presentation of self antigens has potentially far-reaching significance in self-tolerance, autoimmunity, and alloreactivity.

Mta is an immunological mystery. On one hand, Hmt, which belongs to a group of "nonclassical" MHC class I molecules that are not known to be antigen-presenting molecules, restricts T cell recognition of Mtf peptides. On the other, Mtf peptides are not restricted by the H-2K/D/L antigen-presenting molecules, which are the restriction elements of all other defined conventional antigens (20). To account for this unusual pattern of Mta T cell recognition, we postulated that the Hmt gene product would have a special chemical affinity for Mtf peptides (20). In addition, Mtf peptides should have distinct biochemical properties that prevent or limit their ability to be restricted by H-2K/D/L antigen-presenting molecules. Because prokaryotic and mitochondrial ribosomes, but not cytoplasmic ribosomes, initiate proteins with N-formyl-methionine (36), we hypothesized that the N-formyl group on mitochondrial peptides might provide the requisite chemical tag (20). We sought to test our model by asking whether the N-formyl group was required for synthetic Mtf peptide activity, and whether this moiety was recognized by specific antigen-presenting molecules on target cells.

Materials and Methods

Mice. F1 mice of (NZB F × BALB/c O; Mta\textsuperscript{a}), and (B10.D2 F × NZB O; Mta\textsuperscript{a}) were bred in our colony from breeding stocks from The Jackson Laboratory (Bar Harbor, ME). B10.CAS2 (Mta\textsuperscript{a}) mice were bred in our colony from stocks provided by Dr. J. Klein, Tübingen, FRG.

MLC and CTL Clones. As described previously (24, 37), F1 mice were primed by intraperitoneal injection of 4 × 10\textsuperscript{7} splenocytes from Mta-disparate reciprocal F1 male mice. After 3 wk, the spleen cells of the responding mice were cultured in supplemented Mishell-Dutton medium (sMDM) for 3–6 d with γ-irradiated (1,500 rad) splenocytes from the immunizing strain at a 6:1 responder-to-stimulator cell ratio. At the end of the initial culture, viable cells were plated at limiting dilution (1, 10, or 1,000 cells/well) in 96-well flat-bottomed culture plates with γ-irradiated stimulator cells and an ammonium sulfate-purified supernatant from PMA-induced EL4 cells, containing 10 U/ml IL-2 (37). Cultures were fed weekly for 2–4 wk with fresh sMDM/IL-2 and stimulator cells (4–6 × 10\textsuperscript{6} cells/ml). Cultures from the microtiter wells were screened for their ability to lyse \textsuperscript{51}Cr-labeled Con A blasts (CABs)
from the stimulating strain. Positive wells were expanded to 24-well plates (Linbro, McLean, VA) and screened for specificity on \(^{51}\)Cr-labeled CABs from Mta-disparate, reciprocal F1 strains. Cultures demonstrating Mta\(^a\) specificity were maintained by weekly seeding \(3 \times 10^4\) viable CTLs into 1.5 ml of fresh sMDM/IL-2 with irradiated stimulator cells \((3-4 \times 10^6\) cells/ml) in a 24-well plate. Most of the lines were derived from limiting dilution cultures that were seeded at an average of 1 or 10 cells/ml, in which fewer than 20% of the wells were positive for cytotoxic activity.

**Target Cells.** Target cells were from the tumor cell lines EL4 (C57BL/6 thymoma, H-2\(^b\), Mta\(^c\)) and WEHI-105.7 (NZB thymoma, H-2\(^d\), Mta\(^b\)) or were lymphoblasts harvested 3-5 d after culture with Con A. CABs were generated as described by Smith et al. (38) by culturing \(5 \times 10^5\) splenocytes/ml for 3-5 d in modified Mishell-Dutton medium (mMDM) with 2.5 \(\mu g/ml\) Con A (Pharmacia Fine Chemicals, Uppsala, Sweden). The tumor cell lines EL4 and WEHI-105.7 were maintained in DME with 10% FCS. Target cells at \(10^6\) cells/ml in DME + 10% FCS were incubated for 12-15 h (except where indicated) with the appropriate peptide at a final peptide concentration of 400 nM and 0.5% DMSO (required because of the hydrophobicity of the peptides). The cells were centrifuged, labeled with 150 \(\mu Ci\) of \(^{51}\)Cr for 60-75 min at 37°C, and washed in cold HBSS containing 5% FCS. The labeled cells were then washed twice and resuspended at \(5 \times 10^5\) cells/ml in assay medium (supplemented MEM, 10% FCS, and 50 \(\mu g/ml\) gentamicin).

**Peptide Synthesis.** Peptides in Table I were synthesized by solid-phase techniques on a peptide synthesizer (No. 430A; Applied Biosystems, Inc., Foster City, CA) using the small scale rapid cycle chemistry programs from Applied Biosystems. \(\text{t-Boc} - \text{t-Boc}\)-protected amino acids were coupled to the \(\text{t-Boc}\) amino acid-\(\text{OCH}_2\)-phenyltoanilidomethyl-polystryrene resin. All amino acids and loaded resins were purchased from Applied Biosystems except the formylated and acetylated methionine, formylated valine, and formylated phenylalanine which were from Bachem Bioscience Inc. (Philadelphia, PA). Peptides were cleaved from the resin and deprotected using trifluoroacetic acid-trifluoromethanesulfonic acid procedure (39). After cleavage the peptides were precipitated and washed with anhydrous ether, dried, and solubilized in DMSO. Peptides were assayed for purity by reverse-phase HPLC and amino acid analysis (PicoTag System; Waters Associates, Milford, MA); several peptides were also analyzed by NH\(_2\)-terminal amino acid sequence analysis (477A Protein Sequencing System, Applied Biosystems, Foster City, CA).

**Competition Experiments.** WEHI-105.7 cells at \(10^6\) cells/ml were incubated at 37°C with increasing concentrations of fMet\(6\)-\(\alpha\), AcMet\(6\)-\(\alpha\), Met\(6\)-\(\alpha\) (peptides, IV, V, and VIII; Table I) or the chemotactic peptides N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys, N-formyl-Met-Leu-Phe-Phe, and N-formyl-Met-Leu-Phe; or the chemotactic antagonist t-Boc-Met-Leu-Phe (Sigma Chemical Co., St. Louis, MO). After 4-10 h, a 20 nM concentration of fMet\(12\)-\(\alpha\) (peptide II, Table I) was added to each mixture and the cells were reincubated for another 4-10 h. Target cells were then labeled with \(^{51}\)Cr, washed, and processed as above.

**CML Assay.** As described previously (24), long-term monoclonal CTLs were collected, washed once, and resuspended in assay medium. Viable CTLs (effector cells) were counted by fluorescein diacetate uptake. 100 \(\mu l\) of CTL suspension were added to 96-well round-bottomed culture plates (Costar, Cambridge, MA). Target cells at 0.5-1.0 \(\times 10^4\) cells per well were plated at multiple effector-to-target cell ratios. After a 4-h incubation at 37°C, the supernatants were harvested using a supernatant collection system (Skatron, Sterling, VA). Spontaneous release (SR) was measured by incubating target cells in assay medium alone; maximal release (MR) was measured from target cells lysed by 1% SDS. Data are expressed as follows: percent specific lysis = \(\frac{[(\text{experimental release} - \text{SR})/(\text{MR} - \text{SR})] \times 100}{\text{MR}}\). The standard error was estimated by propagation of errors. If no error bar is shown, the error was <5%.

## Results

**Exogenous NDI-\(\alpha\) Peptide Mimics Endogenous Mtf\(^a\) Peptide.** To assess the possible role of the N-formyl group in Mta, we established conditions under which exoge-
## Table I

**Synthetic ND1-α and ND1-β Peptides and Analogues**

<table>
<thead>
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<th>Peptide</th>
<th>1</th>
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<th>20</th>
<th>26</th>
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<tr>
<td>I  fMET26-α</td>
<td>FM</td>
<td>FF</td>
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<td>LI</td>
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<tr>
<td>II fMet12-α</td>
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<td>XI fMet26-β</td>
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<td>XII fMet12-β</td>
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nous synthetic NDI peptides were presented appropriately to Hmt-restricted, Mtf-specific CTL. EL4 cells (Mta\textsuperscript{a}) or WEHI-105.7 cells (Mta\textsuperscript{b}) were incubated with a 400 nM concentration of fMet6-α or fMet26-β (peptides I and XI, Table I) and tested for lysis by Mta\textsuperscript{b}-specific or Mta\textsuperscript{a}-specific CTLs, respectively. As expected, polyclonal Mta\textsuperscript{b}-specific CTLs lysed Mta\textsuperscript{a} cells only when incubated with the fMet6-β peptide, not when incubated with the fMet26-α peptide (Fig. 1A). Reciprocally, Mta\textsuperscript{a}-specific polyclonal CTL as well as 32/32 CTL clones (8 representative clones are shown) lysed Mta\textsuperscript{b} target cells incubated with fMet26-α, but not with fMet26-β (Fig. 1, B and C). In the specificity assay screened at a single effector-to-target cell ratio, a few Mta\textsuperscript{a}-specific clones did exhibit up to 20% lysis on targets pulsed with inappropriate peptide (Fig. 1C); this apparently inappropriate activity was not observed when Mta\textsuperscript{a}-specific CTL clones were expanded and assayed at multiple ratios. Additionally, neither cell line lost its native antigenic phenotype when incubated with the disparate peptide (data not shown).

**Figure 1.** NDI-α and β peptides mimic endogenous Mtf gene products α and β. The indicated NDI peptides (fMet26-α or fMet26-β) were incubated with EL4 (Mta\textsuperscript{a}) or WEHI 105.7 (Mta\textsuperscript{b}) target cells at a concentration of 400 nM in 0.5% DMSO final concentration. Incubation was for 12 h at 37°C before ⁵¹Cr labeling. The cells were washed four times before inclusion in a 4-h ⁵¹Cr-release assay. Control cells were similarly incubated in 0.5% DMSO alone. (A) Specific recognition of NDI-β (fMet26-β) peptide by polyclonal Mta\textsuperscript{b}-specific CTLs. Polyclonal CTL were generated by immunizing Mta\textsuperscript{b} (B10.D2 ♀ × NZB ♂) F1 mice with NZB (Mta\textsuperscript{a}) spleen cells. (B) Specific recognition of NDI-α (fMet26-α) peptide by polyclonal Mta\textsuperscript{a}-specific CTLs. Polyclonal CTL were generated by immunizing Mta\textsuperscript{a} (NZB ♀ × BALB/c ♂) F1 mice with BALB/c (Mta\textsuperscript{a}) spleen cells. (C) Specificity of selected Mta\textsuperscript{a}-specific clones for recognition of NDI-α peptide. CTL clones were assayed at a 10:1 ratio of effector-to-target cells.
**NDI-α Peptide Recognition Is Hmt Restricted.** CTL-mediated immune recognition of native (21) and tunicamycin-induced (37) forms of Mta are all Hmt restricted for recognition by T lymphocytes. If exogenous NDI peptides sensitize target cells to antigen-specific cell-mediated cytolysis, peptide recognition should also be Hmt restricted. We tested this hypothesis using CABs from B10.CAS2 mice, which carry the functionally null Hmt b allele. B10.CAS2 splenocytes are resistant to lysis by Mta a-specific CTLs even though these mice express endogenous Mtf a (20, 21). Preincubation of Hmt a but not Hmt b CABs with a 400 nM concentration of either fMet26-α or fMet26-β (peptides I and XI; Table 1) induced susceptibility to lysis by Mta a-specific CTLs. We conclude that NDI-α peptide recognition is Hmt restricted (Fig. 2).

**Hmt Can Restrict a Peptide as Short as Six Amino Acids.** To define the minimal structure of the Mtf a product recognized by Mta a-specific CTLs, a set of four peptides of decreasing length were synthesized (peptides fMet26-α, fMet12-α, fMet8-α, and fMet6-α; Table 1). Using these peptides, which differed only in length at their COOH-termini, we observed that polyclonal CTLs lysed target cells pulsed with fMet26-α and fMet12-α peptides much more efficiently than they did targets incubated with fMet8-α (Fig. 3 A). No lysis was observed when the fMet6-α was used (Fig. 3 A). In contrast, all 32 Mta a-specific CTL clones recognized α-fMet8 as well, or nearly as well, as they did fMet26-α and fMet12-α. Surprisingly, 8 of 32 clones lysed Mta b targets pulsed with the shortest peptide tested, fMet6-α (e.g., clones 1B3, 1G11, and 3D5; Fig. 3 B). This exquisite discrimination was critical to competition experiments described below.

**The N-formyl Group in NDI-α Peptides Is Required for Mtfα Mimicry.** We proposed that Mtf peptides should have distinct biochemical properties to account for its selective restriction by Hmt. To test the hypothetical requirement for the N-formyl group in NDI peptides, two analogues of fMet12-α were synthesized (peptides V and VII; Table 1), containing either nonsubstituted methionine (Met12-α) or N-acetylated methionine (AcMet12-α) at the NH2 terminus. Neither the nonsubstituted peptide nor the acetylated analogue induced recognition and lysis of Mta b target cells by Mta a-specific CTLs. The requirement for the formyl group at the NH2 terminus of NDI-α was observed both with Mta a-specific polyclonal CTL and with all 32 Mta a-specific CTL clones (Fig. 4).
Figure 3. Polyclonal and monoclonal CTL recognition of decreasing length of ND1-α. WEHI 105.7 targets (Mta<sup>b</sup>) were pretreated with a 400 nM concentration of the designated peptides, <sup>51</sup>Cr-labeled, and washed four times before addition to a 4-h <sup>51</sup>Cr-release assay. (A) Mta<sup>a</sup>-specific polyclonal CTL recognition of <sup>51</sup>Cr-labeled WEHI-105.7 cells (Mta<sup>b</sup>) preincubated with equimolar concentrations of fMet26-α, fMet12-α, fMet8-α, or fMet6-α, or with DMSO alone. (B) CML assays with Mta<sup>a</sup>-specific clones at 10:1 ratio of effector-to-target cells.
The Effect of Position 1 Methionine Variation on ND1-α Recognition. Because the N-formyl group of the initiator methionine was essential for Mtaα recognition, we reasoned that substituting methionine with other hydrophobic amino acids might interfere with Mta-specific immune recognition. To test this idea, two analogues were synthesized with formyl-phenylalanine (Whe12-α) or formyl-valine (Val12-α) at the same position (peptides IX and X; Table I). Using polyclonal Mtaα-specific CTLs, the specific lysis of targets incubated with equimolar concentrations of these analogues (Whe12-α and Val12-α) was reduced by 50–75%, as shown in Fig. 5A. In contrast, most of the Mtaα-specific clones recognized the formylated analogs as efficiently or nearly as efficiently as they recognized fMet12-α (e.g., clones 1E3 and 1D3; Figure 5B); however, some were less efficient (e.g., clones 3D5 and 1C5). Thus, although the NH2-terminal formyl group was absolutely required for the immune recognition of Mta, the identity of the NH2-terminal amino acid was not critical for 28/32 CTL clones. These findings suggested that the N-formyl amino acid interacts specifically with the antigen-presenting molecule, and that the Mtf peptide interaction site on Hmt product may be an N-formyl-amino acid binder.

An N-Formyl-Peptide Receptor Is Required for the Expression of Mta. The requirement for an N-formyl group at the NH2 terminus of the ND1 peptide might reflect epitope discrimination by Mta-specific T cell receptors, binding by N-formyl peptide
receptors on the target cell, or both. If the second model is correct, i.e., that target cells bear an N-formyl receptor required for Met product presentation, then nonantigenic N-formyl peptides of the correct molecular shape should compete for binding and interfere with the presentation of exogenous fMet12-α.

To determine the appropriate conditions for competition experiments, we established the time-course and dose-response required to transform WEHI-105.7 cells (Mta\(^b\)) into Mta\(^a\)-like targets. For the dose-response curve, WEHI-105.7 cells were incubated for 12-14 h with concentrations of fMet12-α ranging from 10 to 800 nM (peptide II, Table I), and tested for lysis by Mta\(^a\)-specific CTL clones 1D3 and 1G11. As shown in Fig. 5A, a concentration of fMet12-α peptide as low as 10 nM caused maximum target cell lysis. The time course was established by incubating WEHI-105.7 cells with a 400 nM concentration of fMet12-α for up to 18 h (\(^{51}\)Cr labeling included). 2 h or less were sufficient to cause maximum target lysis by Mta\(^a\)-specific clones (Fig. 5B).

We chose as a potential competitor the fMet6-α peptide, which is antigenic for some Mta\(^a\)-specific CTL clones but not for others. WEHI-105.7 cells (Mta\(^b\)) were incubated with increasing concentrations of fMet6-α, AcMet6-α, or Met6-α (peptides IV, VI, and VIII; Table I) for 4 h before the addition of a 20 nM concentration of fMet12-α. Target cells were then incubated with Mta\(^a\)-specific clones that recognized fMet6-α (e.g., 1G11; Fig. 7B), or did not recognize it (e.g., 1D3; Fig. 7A). Inhibition of target cell lysis was observed with a concentration of fMet6-α peptide
FIGURE 6. Rapid and low-dose ND1 peptide induction of Mta. (A) WEHI 105.7 cells (Mta²) were incubated at 37°C with a 400 nM concentration of fMet12-α for the indicated times (including ⁵¹Cr labeling). Cells were washed four times before incubation in a 4-h ⁵¹Cr release assay at an effector-to-target ratio of 4:1 for CTL clones 1G11 and 1E3. (B) WEHI 105.7 cells were incubated with increasing concentrations of fMet12-α at 37°C for 12 h before ⁵¹Cr labeling. Cells were then washed four times and added to CML assays at an effector-to-target cell ratio of 4:1 for CTL clones 1G11 and 1D3.

FIGURE 7. A novel N-formyl receptor is required for Mta expression. WEHI-105.7 cells were incubated with increasing concentrations of the indicated peptides of ND1-α for 3-4 h before addition of a 20 nM concentration of fMet12-α. The following morning cells were labeled with ⁵¹Cr, washed four times, and incubated with Mta²-specific clones 1D3 (A) or 1G11 (B) before incubation in a 4-h ⁵¹Cr release assay at an effector-to-target cell ratio of 4:1.
as low as 1 μM (50-fold excess) and was maximal with 2 μM (100-fold excess) when clone 1D3 was used as an effector. However, no effect was seen with a 1,000-fold excess of fMet6-α peptide when clone 1G11 was used. Significantly, no inhibition of target cell lysis was observed with either clone 1D3 or 1G11 when AcMet6-α or Met6-α (peptides VI and VIII; Table I) were used as competitors. Our results demonstrate that a receptor critical to Mtf peptide presentation can be competitively occupied by fMet6-α peptide, but not AcMet6-α or Met6-α peptides.

The N-formyl-NDI Peptide Receptor Differs from the Chemotactic Peptide Receptor. To distinguish the Mta-associated N-formyl peptide receptor from the chemotactic peptide receptor, which also binds some N-formyl peptides, we attempted cross-competition experiments. If both receptors are the same, known chemotactic peptides should competitively inhibit the activity of Mtf peptides. We chose three potent chemotactic peptides: N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys, N-formyl-Met-Leu-Phe-Phe, and N-formyl-Met-Leu-Phe; and a chemotactic antagonist t-Boc-Met-Leu-Phe (40-42). As shown in Table II, all four peptides failed to inhibit target lysis (IC50 of >20 μM). In contrast, the IC50 of fMet6-α was <1-3 μM. In addition, incubating WEHI-105.7 cells with up to 20 μM of any of these chemotactic peptides did not render these cells susceptible to lysis by any Mta-specific CTLs (data not shown). These results demonstrate that the Mta-associated N-formyl receptor is distinct from the chemotactic formyl-peptide receptor.

Discussion

Our results demonstrate a requirement for the N-formyl group of Mtf peptides for target cell recognition by Mta-specific CTLs. In addition, our competition experiments establish that a novel N-formyl peptide receptor expressed on APCs is required for Mta expression. Although we do not know the identity or the characteristics of this receptor, we can speculate on candidates. With the exclusion of the chemotactic receptor (43, 44), there are at least two possible candidates for the Mta-associated N-formyl receptor at the surface of the APC: the Hmt gene product, or

| Table II |
| Mta-associated N-Formyl Peptide Receptor Is Distinct from the Chemotactic Receptor |

<table>
<thead>
<tr>
<th>Mta*-Specific clone</th>
<th>Exp.</th>
<th>fMet6-α</th>
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<th>fMLPP</th>
<th>fMLP</th>
<th>t-Boc-MLP</th>
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WEHI 105.7 cells were incubated with increasing concentrations (0-20 μM) of the chemotactic peptides fNle-Leu-Phe-Nle-Tyr-Lys (CTP6), fMet-Leu-Phe-Phe (fMLPP), fMet-Leu-Phe (fMLP); the chemotactic peptide antagonist t-Boc-Met-Leu-Phe (t-Boc-MLP); or fMet6-α. Competition experiments were as described in Fig. 7. The effector-to-target cell ratio was 4:1 for Exp. 1 and 2.5:1 for Exp. 2. The 50% inhibitory concentration (IC50) was estimated from experiments in which fMet6-α peptide inhibited >50% of the maximum specific lysis.
an independent \( N \)-formyl receptor. We favor the former hypothesis as more parsimonious; it accounts at once for the selective restriction of \( Mtf \) peptides by \( Hmt \).

To account for the large hole in the H-2K/D/L repertoire that fails to restrict \( Mtf \) peptides, we propose that these antigen-presenting molecules are inefficient at presenting very hydrophobic molecules such as signal peptides. In contrast, the \( Hmt \) gene product should contain, in addition to the formyl-peptide binding site, a generally hydrophobic pocket. An independent \( N \)-formyl peptide receptor would have to be an intracellular processor that loads \( Mtf \) peptides onto \( Hmt \) gene product. We would expect that such a protein processor would equally load onto H-2K/D/L antigen-presenting molecules. Although the Mta-associated \( N \)-formyl receptor is on the APC and may be the class I molecule itself, we can not exclude the possibility that the \( N \)-formyl group is also recognized by the TCR on the effector cells.

The function of \( N \)-formyl group at the NH\(_2\) terminus of \( Mtf \) peptide could be to neutralize the positive charge at the NH\(_2\) terminus of the peptide, but this argument is not supported by \( N \)-acetylation which did not restore the activity. The requirement for an \( N \)-formyl group has been demonstrated for all functional NDI-\( \alpha \) or -\( \beta \) peptides, irrespective of the peptide length or the first amino acid in these peptides (data not shown). It is clear, however, that other structures in NDI peptides are also required, in that the \( N \)-formylated chemotactic peptides failed to compete for binding similar to fMet6-\( \alpha \).

While the Mta-\( a \)-specific polyclonal CTLs did not recognize the six amino acid peptide from \( Mtf^a \), one-fourth of the long-term CTL clones (8/32) investigated recognized this peptide. In fact, this difference was critical to the competition experiments that established the \( N \)-formyl-NDI peptide receptor. The variation among CTL clones for recognition and lysis of target cells preincubated with NDI-\( \alpha \) peptides of decreasing lengths probably reflects expression of clonotypic T cell receptors. For example, for some cytochrome c-specific T cell clones changes limited to the junctional regions of the TCR sequences altered the specificity for the peptide without altering the MHC specificity (45, 46). Our results on the minimal length of \( Mtf^a \) are compatible with those reported in the literature. Although generally ranging between 9 and 25 amino acids (2, 16), the shortest T cell epitope reported is a pentapeptide from the immediate-early phase regulatory protein of murine cytomegalovirus (13).

Although no direct physical evidence has been presented on binding of NDI peptides to the putative class I-like, \( Hmt \) product, the selective recognition of \( Hmt^a \) cells by the appropriate CTLs implies that \( Hmt \) product is the molecule involved in the interaction with the T cell antigen receptor. It is not understood how the \( Hmt \) product-exogenous \( Mtf \) peptide complex is constructed at the cell surface. Possible mechanisms include exchange of the exogenous \( Mtf \) peptides with the endogenous peptides at the cell surface; association of the exogenous peptide with unoccupied \( Hmt \) molecules at the cell surface; or internalization of \( Mtf \) peptides and intracellular construction of the antigen.

NADH-dehydrogenase is a conserved mitochondrial enzyme composed of about 25 subunits, seven of which (ND1, 2, 3, 4, 4L, 5, and 6) are encoded by the mitochondrial genome (47, 48). ND1 is a remarkably hydrophobic, mitochondrial transmembrane protein of 35 kD (48). It has been proposed that a hydrophobic leader sequence of 11 amino acids may be cleaved by a signal peptidase from Xenopus laevis.
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ND1 after the protein is anchored in the mitochondrial membrane (49). Whether this proposed leader sequence is the native Mtf epitope is still unknown. Although some of the mechanisms of protein import into mitochondria have been well studied (50), the pathways of vectorial transport of mitochondrially encoded proteins are not fully understood (51). However, for the native Mtf peptide to be presented at the cell surface it must be transported via a protein traffic pathway (52, 53). We do not know how native Mtf peptides end up at the cell surface, but it should be possible to study this pathway using drugs that block transport of newly synthesized proteins (54, 55).

The possibility that the Hmt gene product is a scavenger of hydrophobic N-formylmethionyl peptides derived from endogenous mitochondrial translation products has been proposed earlier (20). Further studies will be required to determine whether the Hmt product binds Mtf peptides on the basis of hydrophobicity in addition to the N-formyl group or both. Whether the Mtf product is the only hydrophobic mitochondrial product transported by the Hmt product is not known. Comparing the hydrophobicity profile (56) of the first 20 amino acids from ND1 with the mitochondrial ORFs (47) suggests similar hydrophobic sequences at the NH2 terminus of ND2, ND4, and ND5.

Finally, it is possible that a biological role of the N-formyl peptide receptor may be to select those intracellular peptides derived from organisms and organelles of prokaryotic origin, and present them at the cell surface to the immune system. If so, then the Hmt/N-formyl receptor system might represent an ancestral specialization involved in immune defenses against intracellular prokaryotic pathogens.

Summary

Maternally transmitted factor (Mtf) is a mitochondrial gene that controls the antigenic polymorphism of the MHC class I maternally transmitted antigen (Mta). Synthetic peptides from the NH2 terminus of the mitochondrially encoded NADH dehydrogenase subunit 1 (ND1) mimic Mtf peptide activity in an allele-specific manner. We show that the minimal ND1-α peptide length recognized by Mta-specific polyclonal CTLs was between 8 and 12 amino acids, while some Mta-specific CTL clones recognized a six amino acid peptide. The N-formyl group at the NH2 terminus of ND1 was essential for Mta activity. Competition experiments using N-substituted ND1-α peptides showed that an N-formyl peptide receptor on the target cell, which differs from the chemotactic peptide receptor, was required for Mta expression. The specificity of this receptor can account for the distinct immune restriction of Mta in which Mtf peptides are uniquely restricted by Hmt. It is possible that the Hmt gene product is the N-formyl peptide receptor itself and that it represents a class I antigen presentation molecule specialized for binding, transport, and immune presentation of N-formyl-peptide antigens of mitochondrial and prokaryotic origin.

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References

of New Zealand black mice defined by restriction endonuclease analysis of mitochondrial DNA and by expression of maternally transmitted antigen. J. Exp. Med. 156:2154.


